

Fig. 1 Heparin Sephadex Chromatography

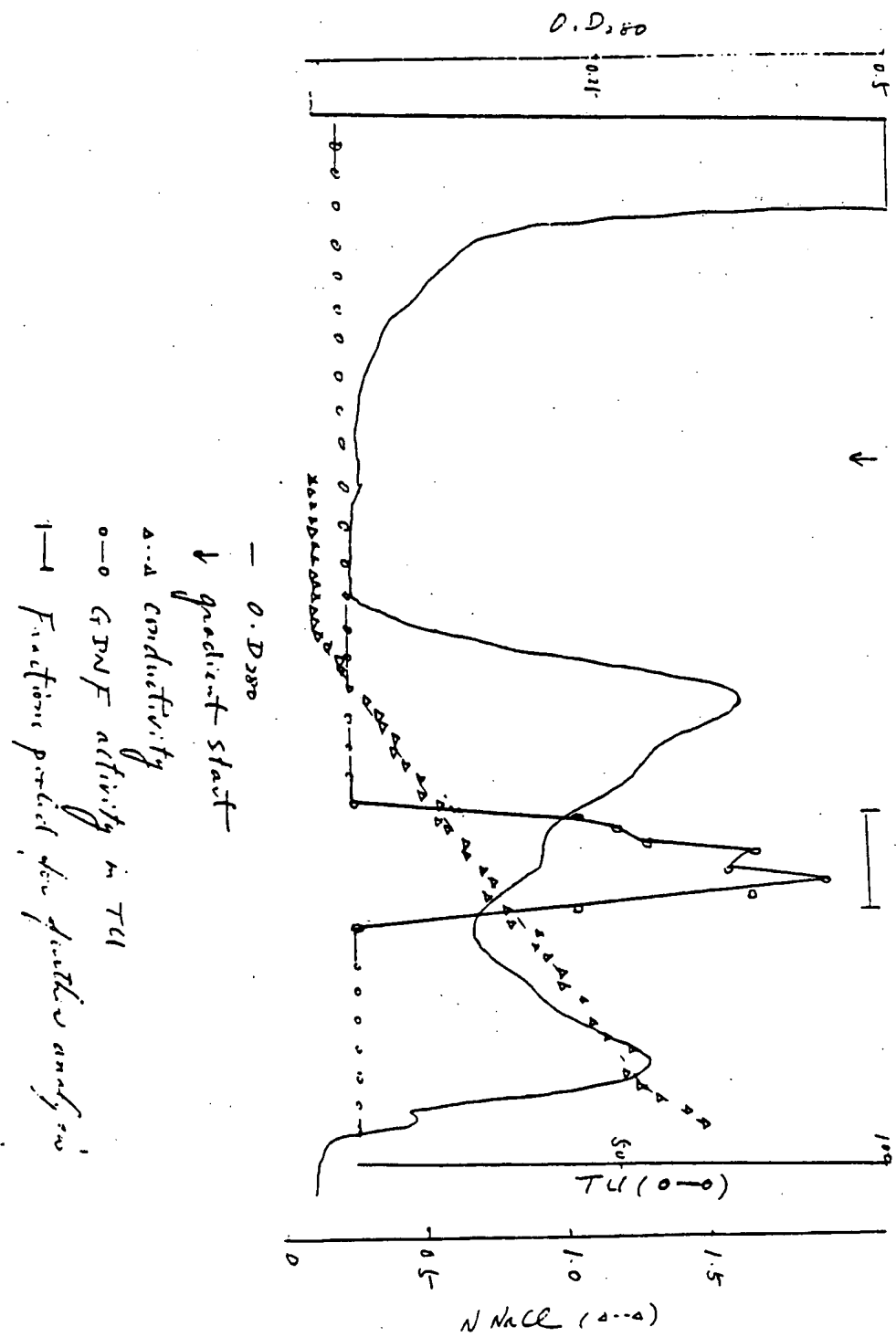
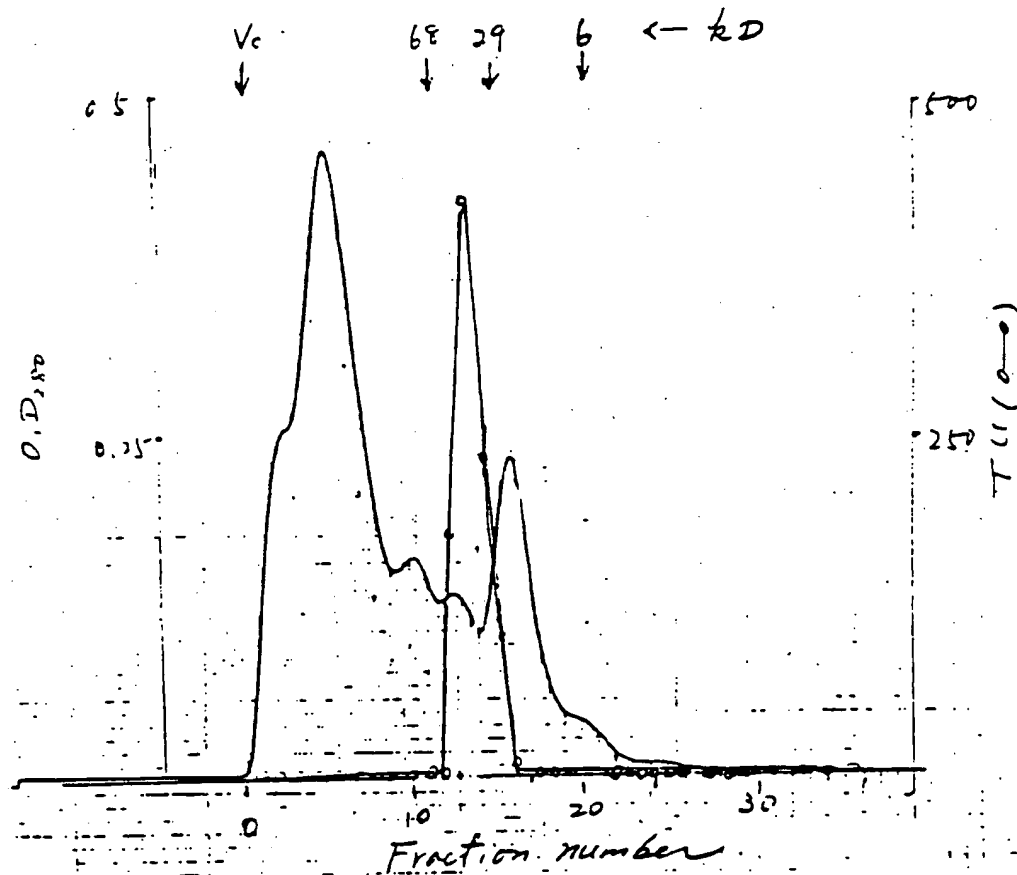


Fig 2 FPLC Superose Chromatography

—  $O.D_{280}$ 

o-o GDNF activity in TU

3/31

Fig. 3. RP-HPLC

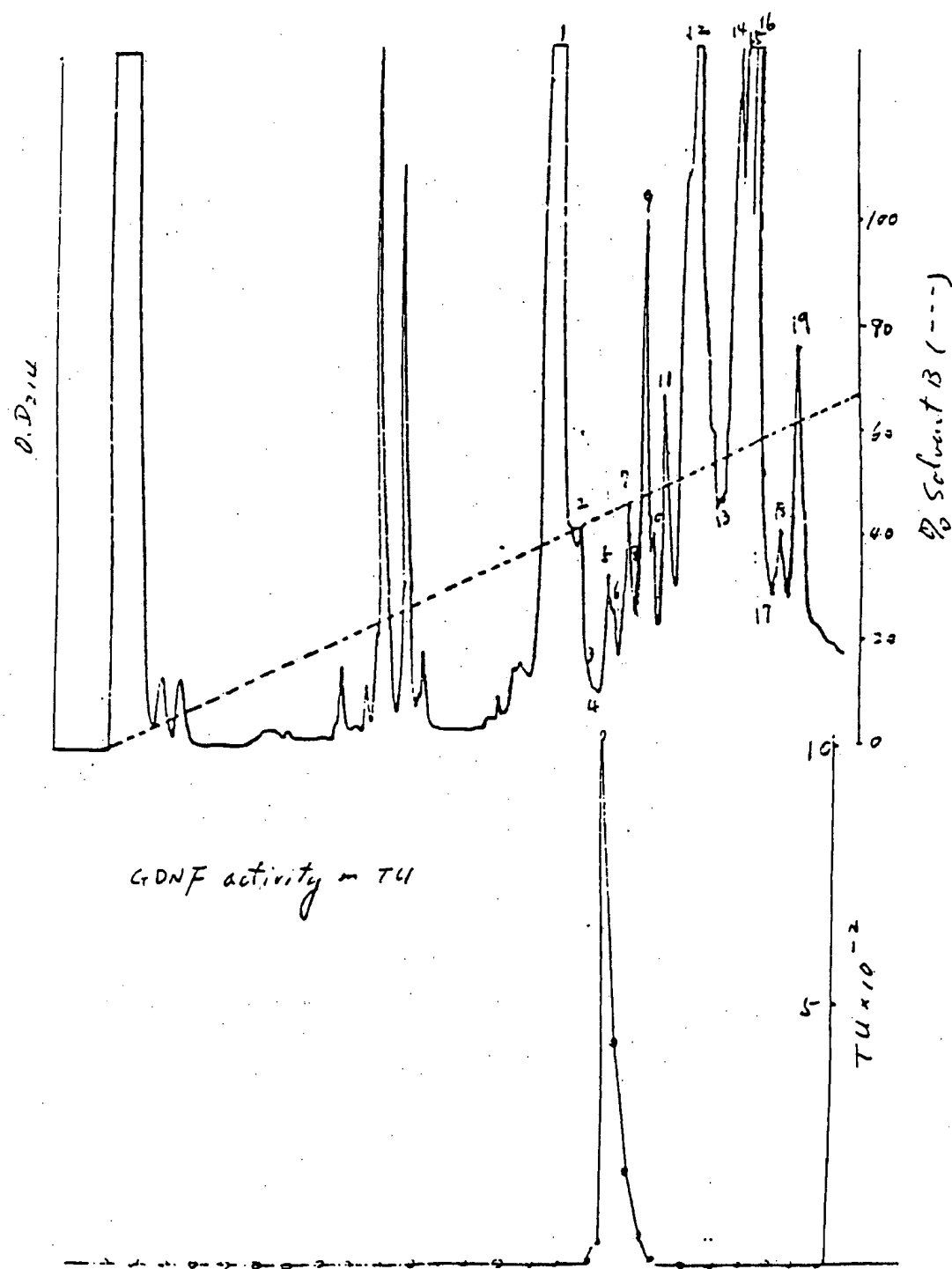
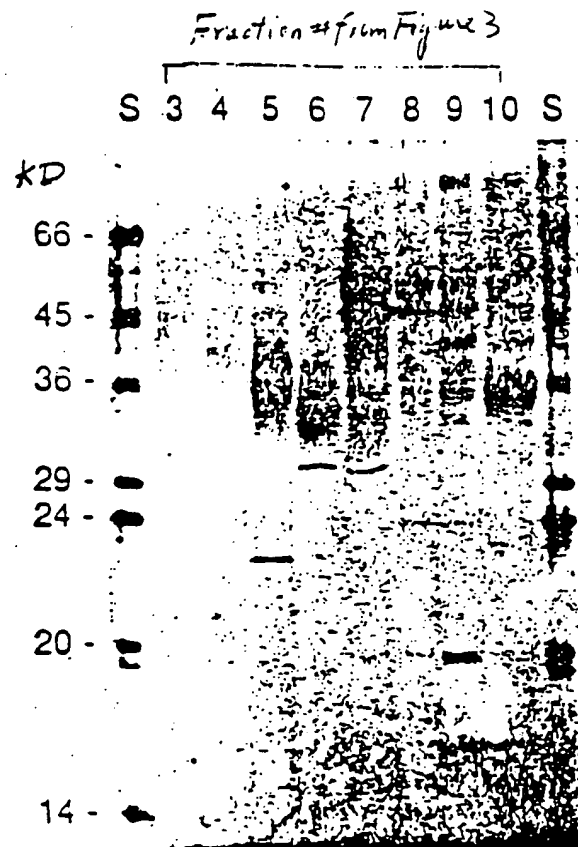
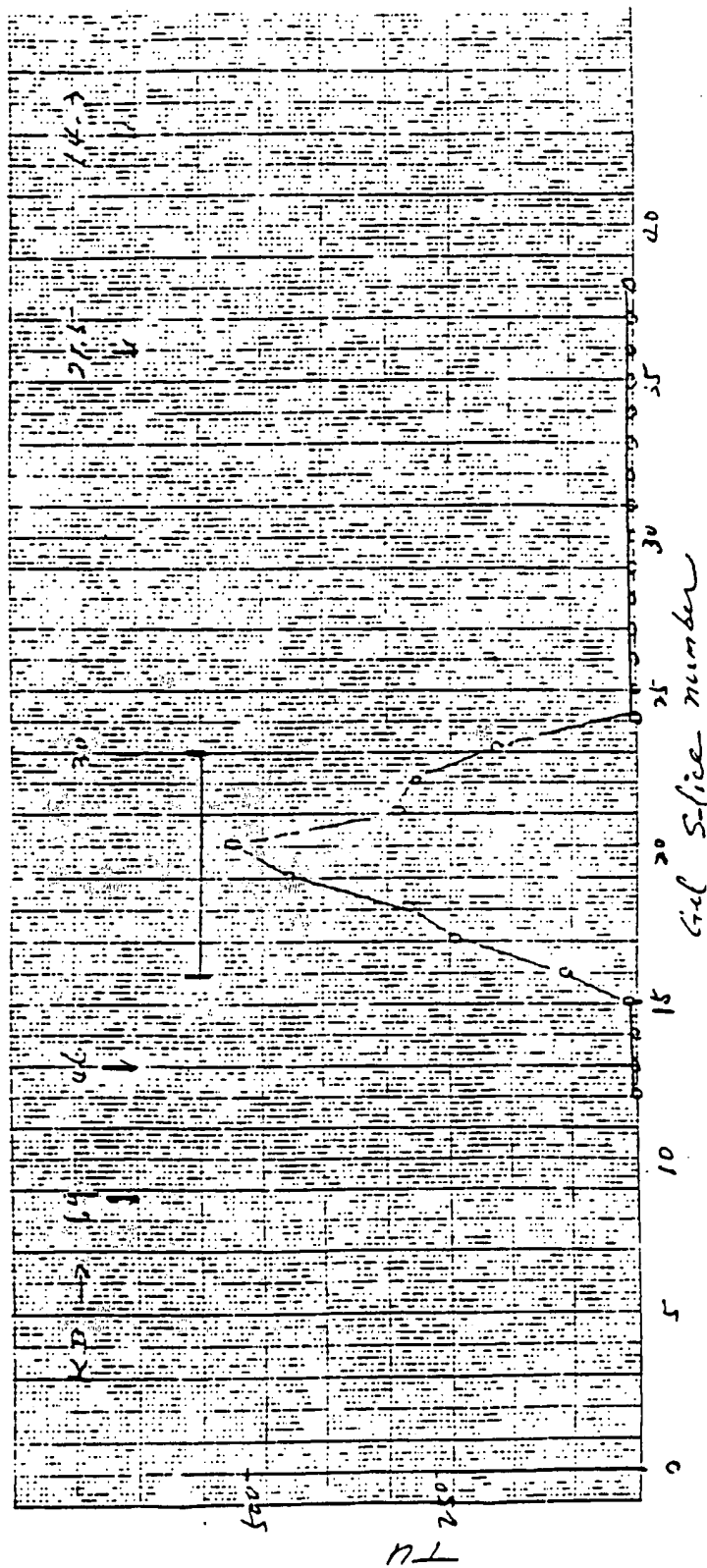


Fig. 4. Silver Stained Nonreducing SDS-PAGE



S: molecular weight standard

Fig 5. GDNF activity off pupative SDS PAGE

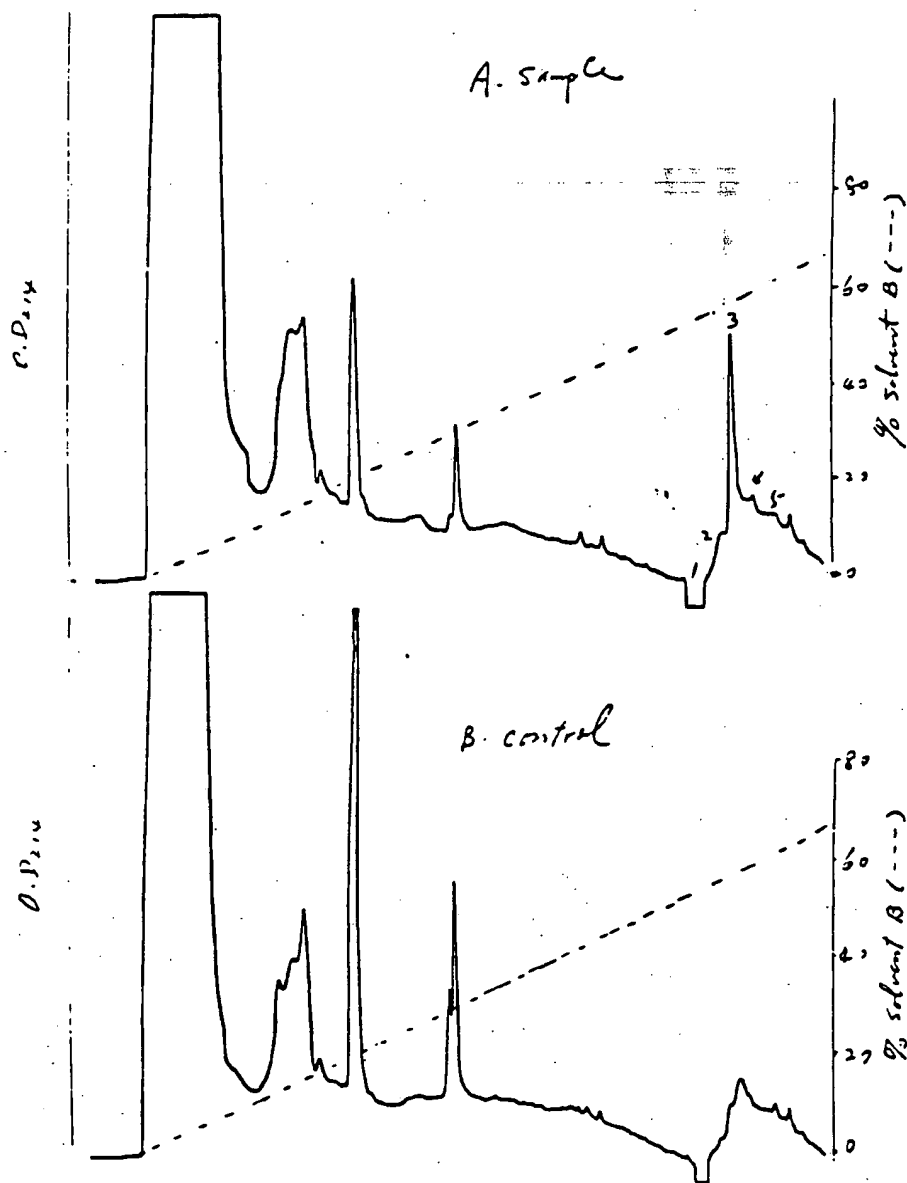


↓ Rainbow protein molecular weight markers (Amersham)

—— Fractions pooled for further analysis

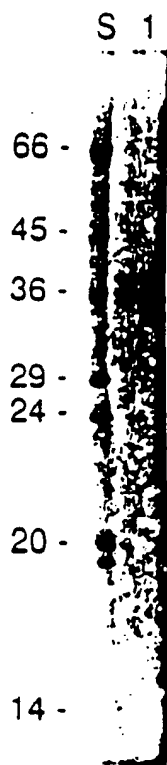
6/31

Fig 6. RP-MPLC of gel slice extract



A. Sample is pooled gel extract from slice #16-23 in Fig 5  
B. Control is pooled gel extract from corresponding slices  
of a blank lane.

Fig. 7 Silver stained non-reducing SDS-PAGE



Lane 1: sample is from peak 3 in Fig. 6A  
 S: molecular weight standard

**FIGURE 8**

Amino-terminal sequence of GDNF

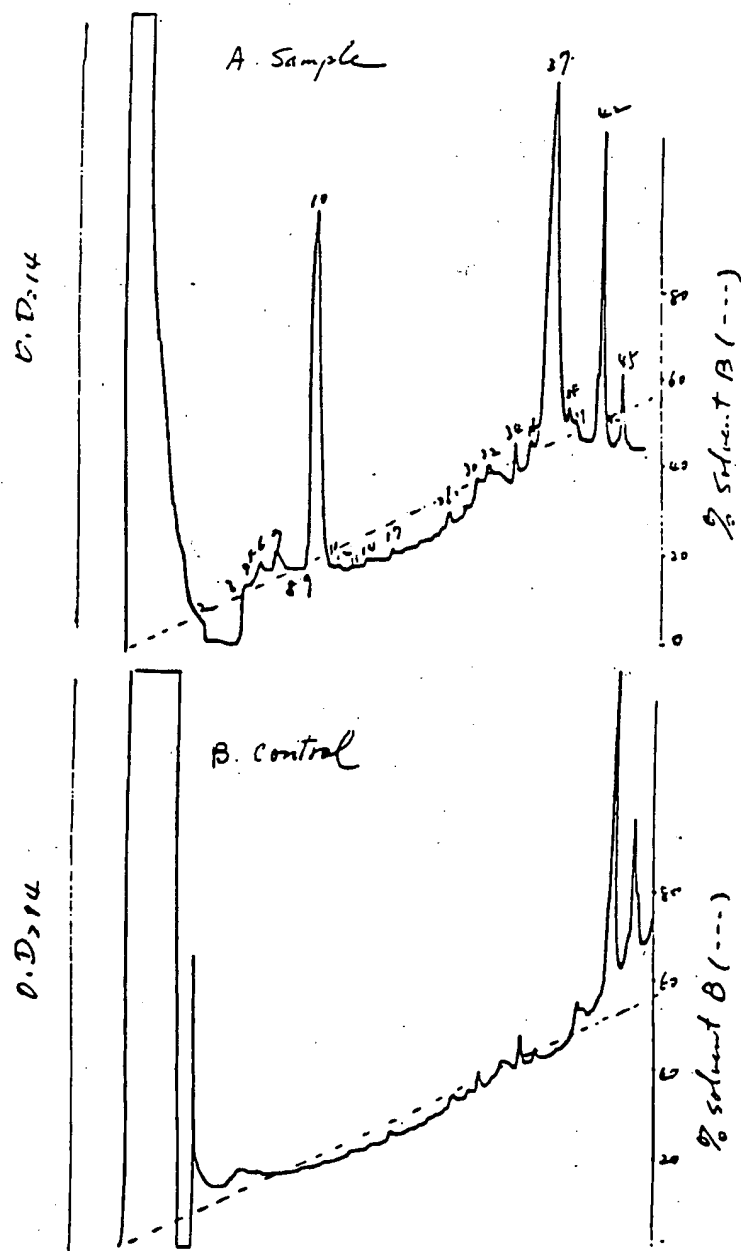
(S)-P-D-K-Q-A-A-A-L-P-R-R-E-(R)-N-( )<sup>\*</sup>-Q-A-A-A-A-(S)-P-  
(D)-(N)

\* no residue could be unequivocally identified in this position

amino acid residues in parenthesis are those identified with less certainty



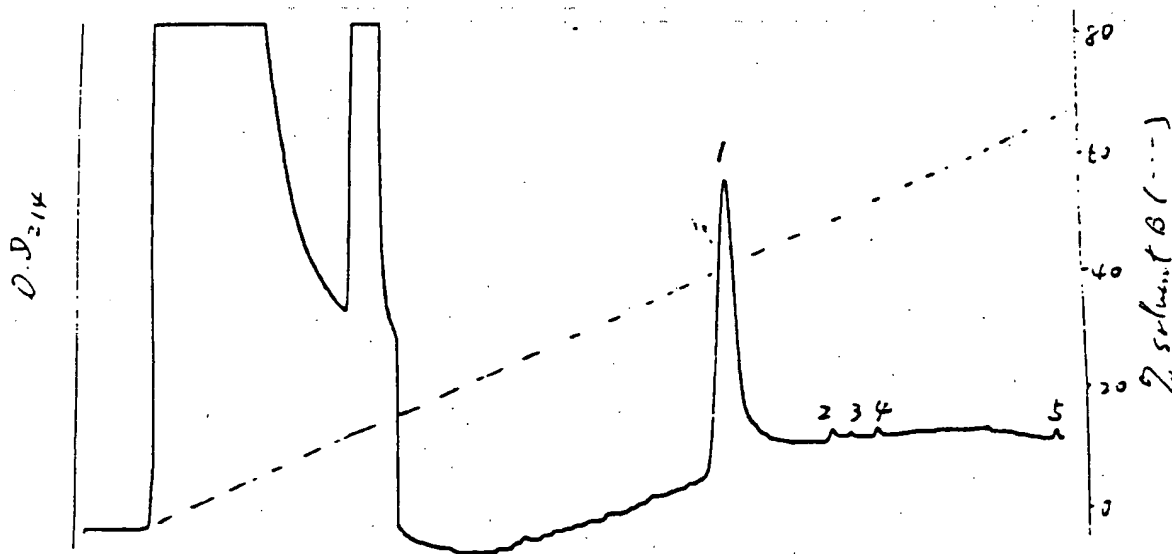
Fig. 7 RP-HPLC of trypsin-digested GRNF



A. Sample is from fractions 5+6 in Fig. 3  
 B. A control contains trypsin only

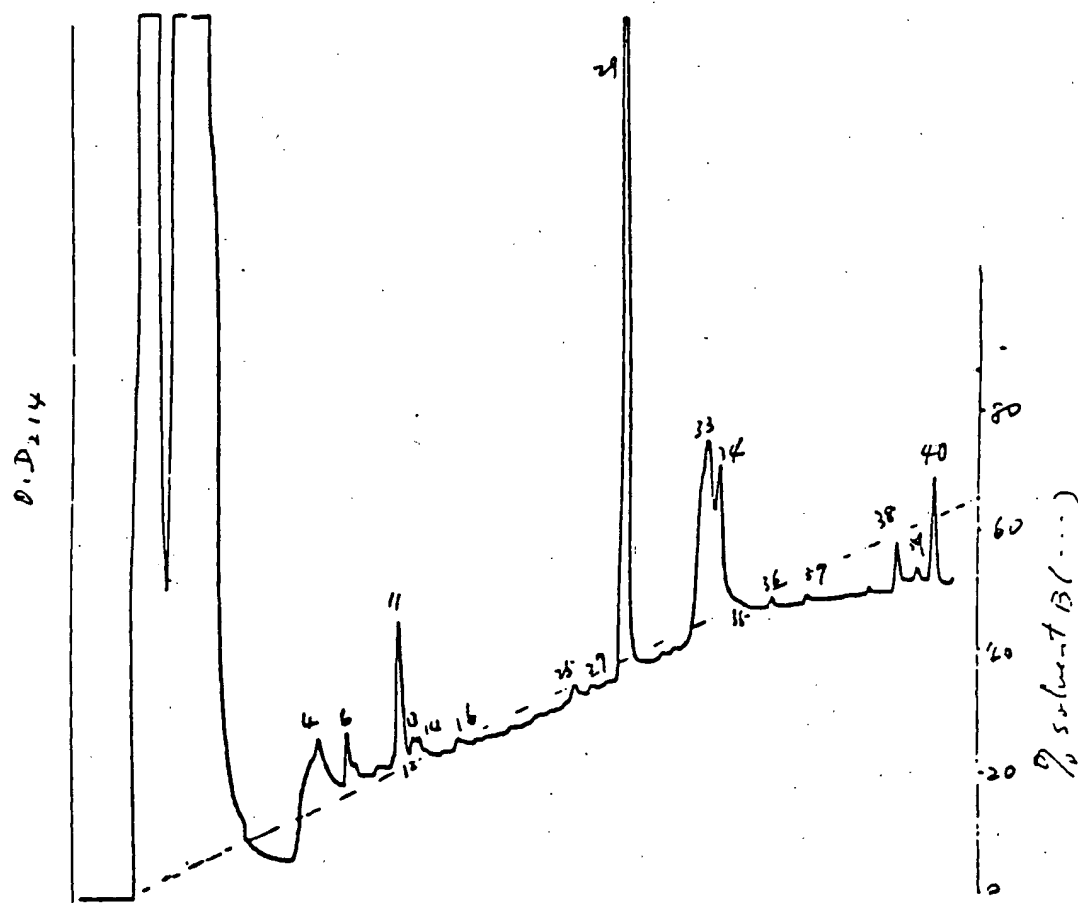
10/31

Fig 10 RP-HPLC of CNBr-treated sample



Sample is from peak 37 in Fig 9A

Fig. 11. RP-HPLC of a reduced CNBr fragment



Sample is from peak 1 in Fig. 10

**FIGURE 12**

An internal sequence of the GDNF

D-K/Q-I-L-K-N-L-(G)\*-(R)-(V)-(R)-(R)-L

\*Amino acid residues placed in parentheses are ones identified with less certainty.

**FIGURE 13**

[illegible]

FIG 13 CONT.

412  
AGA GGG AAA GGT CGC AGA GGC CAG AGG GGC AAA AAT CGG GGG TGC GTC TTA ACT  
R G K G R G R G Q R G G K N R G C V L T

466  
GCA ATA CAC TTA AAT GTC ACT GTC ACT GAC TTG GGT TTT GGC TAC GAA ACC AAG GAG GAA  
A I H L N V T T D L G L G L G Y E T K E E

520  
CTG ATC TTT CGA TAT TGT AGC GGT TCC TGT GAA GCG GCC GAG ACA ATG TAC GAC  
L I F R Y C S G S C E A A E T M Y D

574  
AAA ATA CTA AAA AAT CTG TCT CGA AGT AGA AGG CTA ACA AGT GAC AAG GTA GGC  
K I L K N L S R S R S R R L T S D K V G

628  
CAG GCA TGT TGC AGG CCG GTC GCC TTC GAC GAC GAC CTG TCG TTT TTA GAC GAC  
Q A C C R P V A F D D D L S F L D D

682  
AGC CTG GTT TAC CAT ATC CTA AGA AAG CAT TCC GCT AAA CGG TGT GGA TGT ATC  
S L V Y H I L R K H S A K R C G C I

745

> TGA CCCTGGCTCC AGAGACTGCT GTGTATTGCA TTCCTGCTAC AGTGGGAAGA AAGGGACCAA

Fig. 13 cont.

815

GGTCCCCAGG AATATTTC CCAGAAAGGA AGATAAGGAC CAAGAAGSCA GAGGCAGAGG CGGAAGAAGA

875

AGAAGAAAAG AAGGACGAAG GCAGCCATCT GTGGGAGCCT GTAGAAGGAG GCCCAGCTAC AG

FIGURE 14

S	P	D	K	Q	A	A	A	A	A	A	A	A	A	S	P	E	N	S
L	P	R	R	E	R	N	G	Q	R	Q	R	G	R	G	C	V	L	T
R	G	K	G	R	R	V	T	D	L	D	L	G	Y	E	T	K	E	E
A	I	H	L	N	Y	C	S	G	S	G	S	E	A	E	T	M	Y	D
L	I	F	R	Y	C	S	S	R	S	R	S	R	L	S	D	K	V	G
K	I	L	K	N	L	S	R	R	S	R	S	R	L	S	D	K	V	G
Q	A	C	C	R	P	V	A	A	F	D	D	D	L	S	F	L	D	D
S	L	V	Y	H	I	L	R	R	K	H	S	A	K	R	C	G	C	I



FIGURE 15

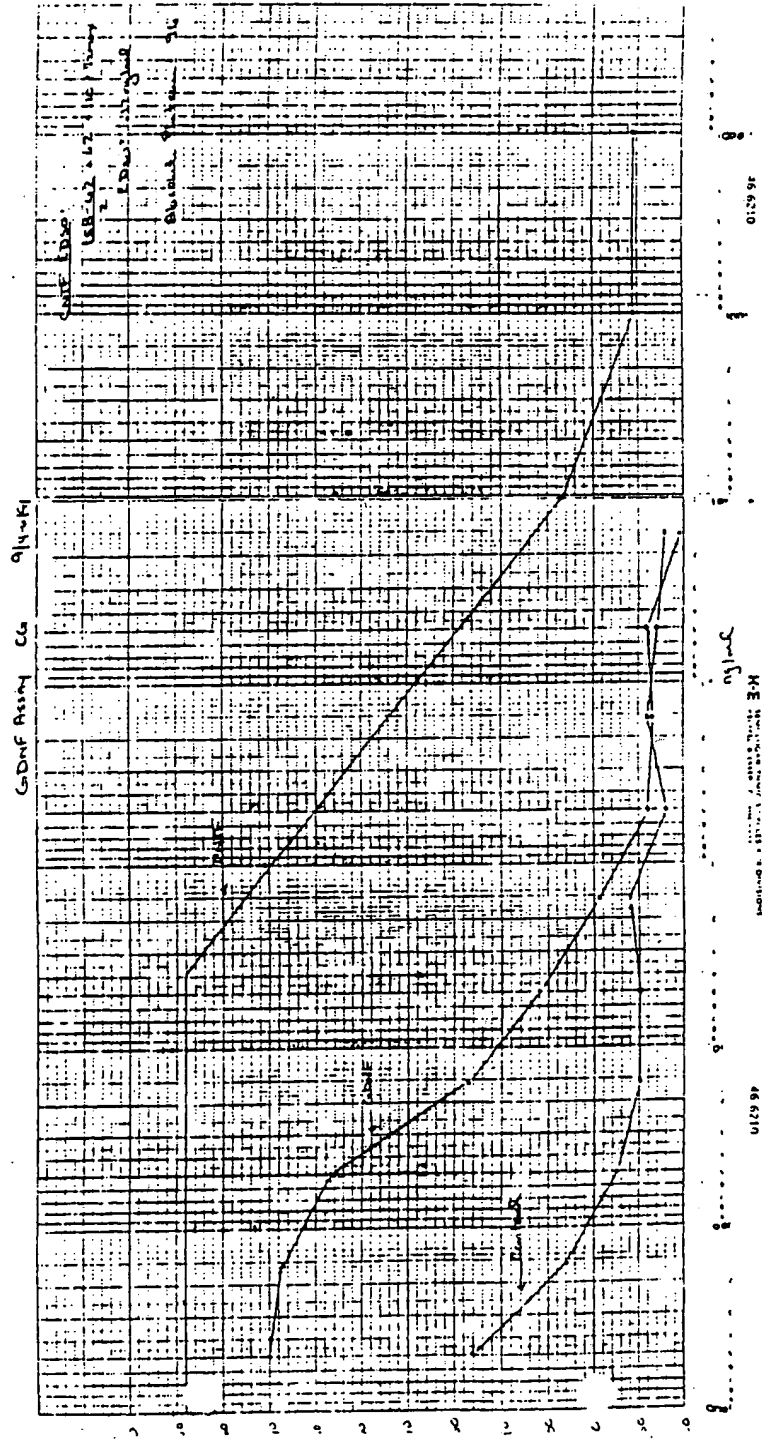


FIGURE 16

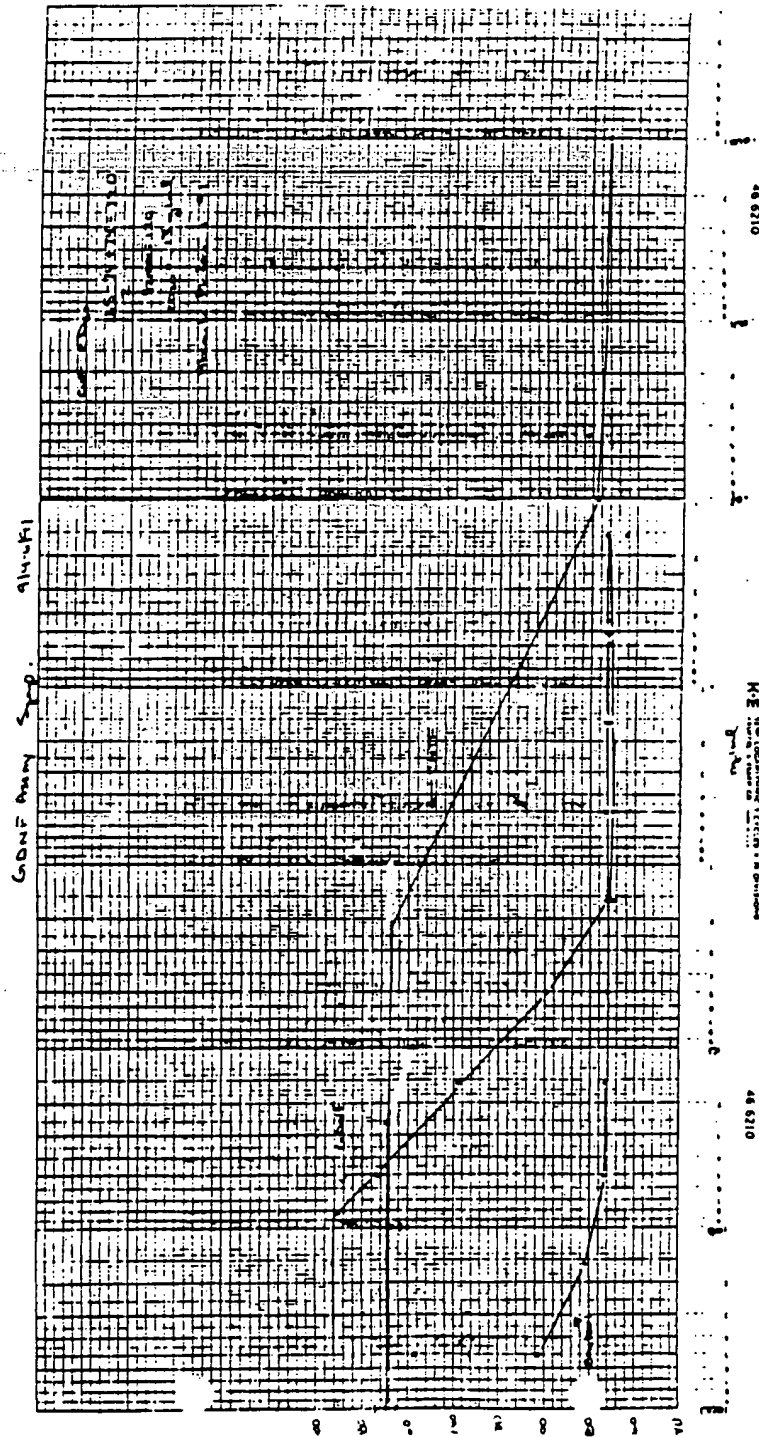
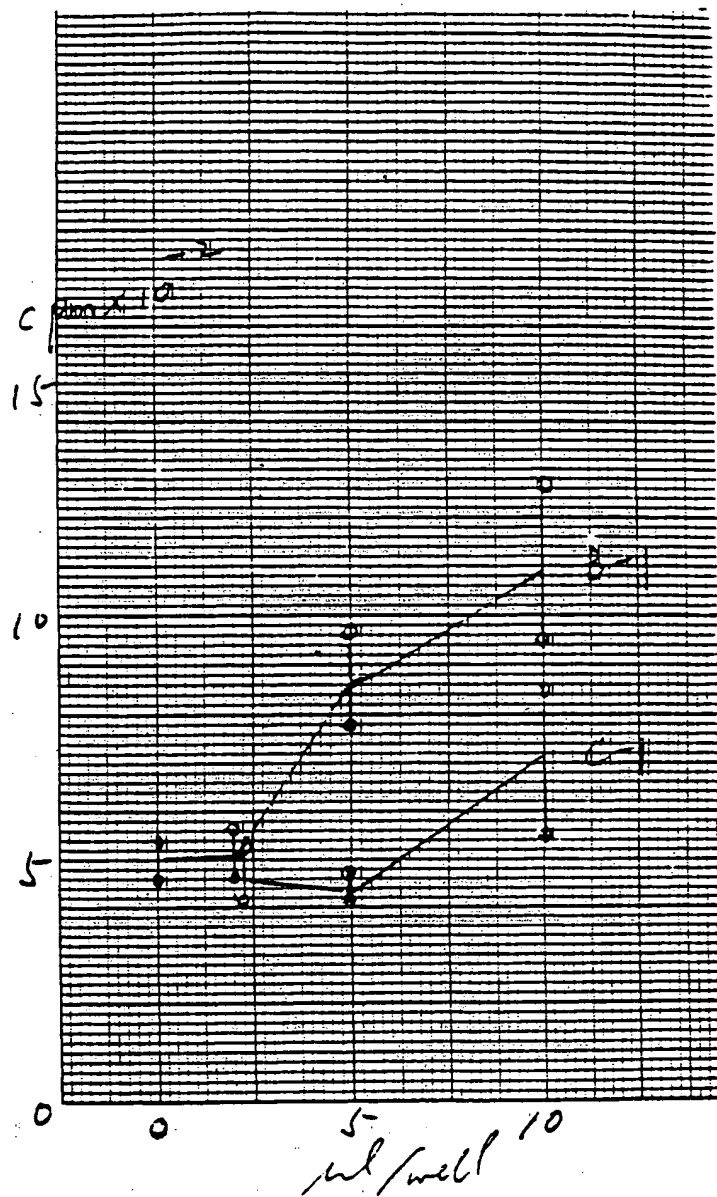


Figure 19/31 17





**FIGURE 19**

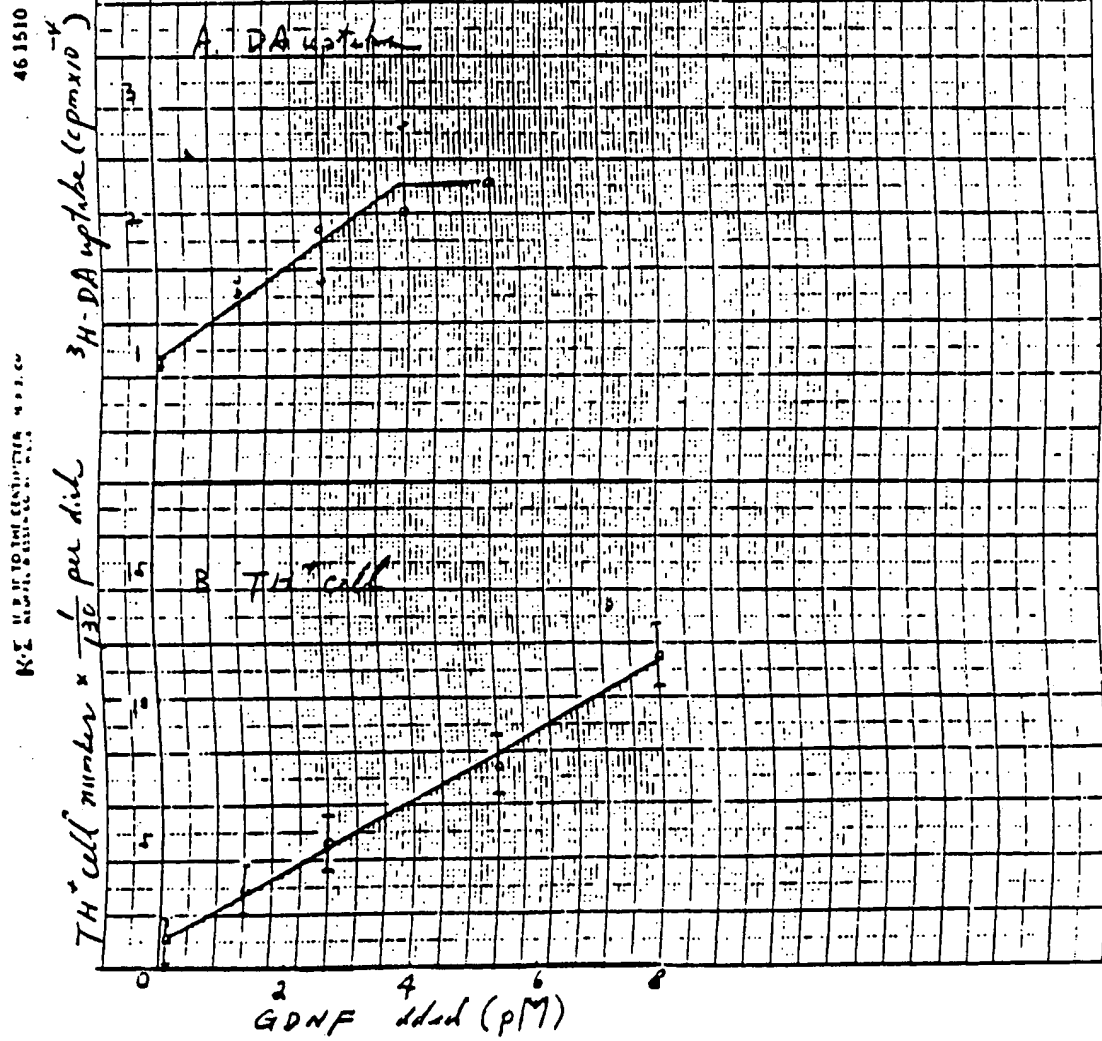
[illegible]

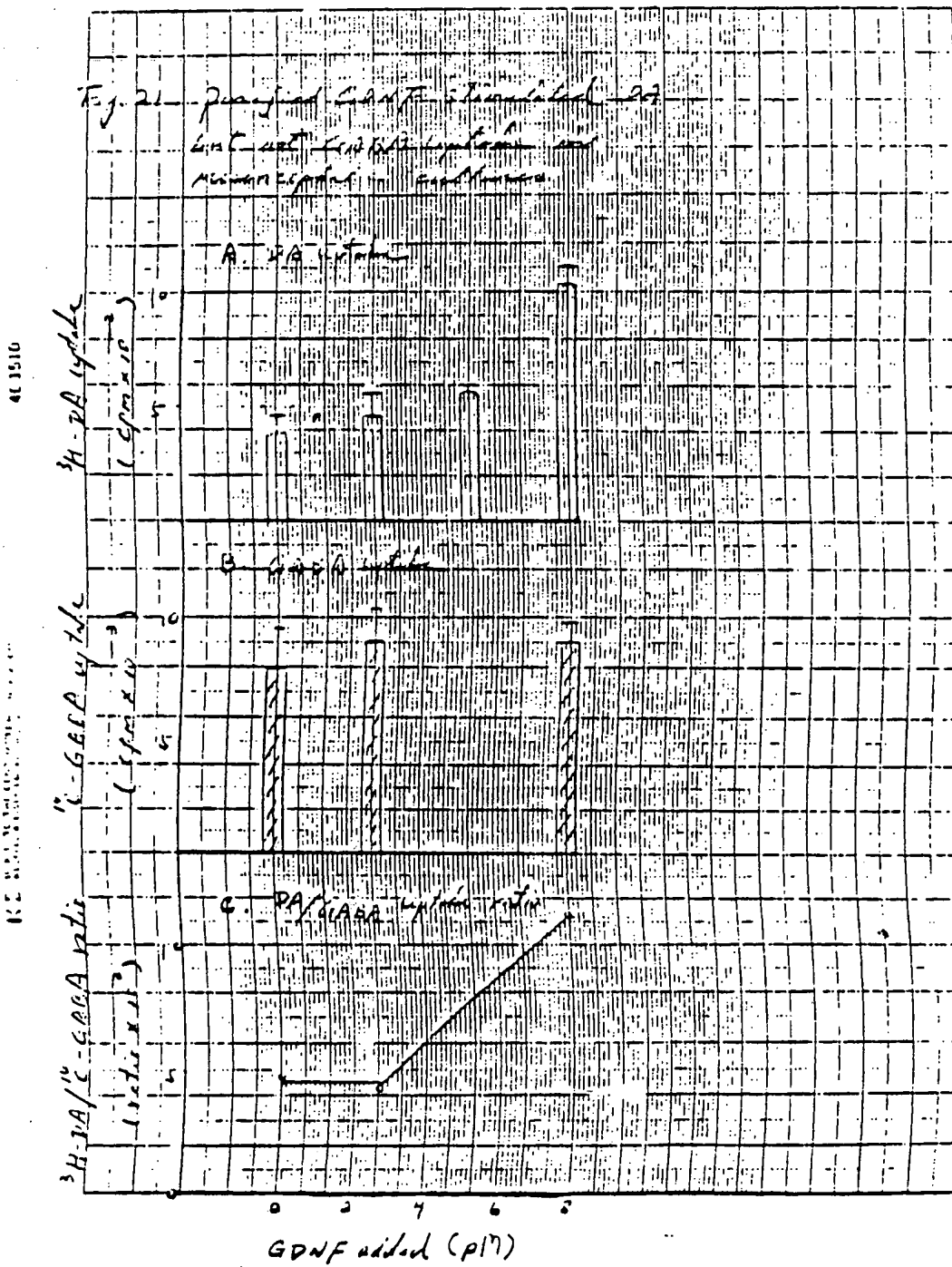
FIG. 19 CONT.

GAT CAT AAC CTG GTT TAC CAT ATT CTA ACA AAG CAT TCC CCT AAA ACG TGT CGA TGT ATC TCA  
 D D N L V Y H I L R K H S A K R C G C I

> ctccggctccagagagactgctgctgattgcattcctgctacagtgcagaagaag

Fig 20 Purified GDNF stimulated DA uptake  
and TH<sup>+</sup> neuronal counts in  
mesencephalic cultures







ttttcccccaactccccgctgcccgcga gGT GCC GCC GCC GCC  
G A A A A

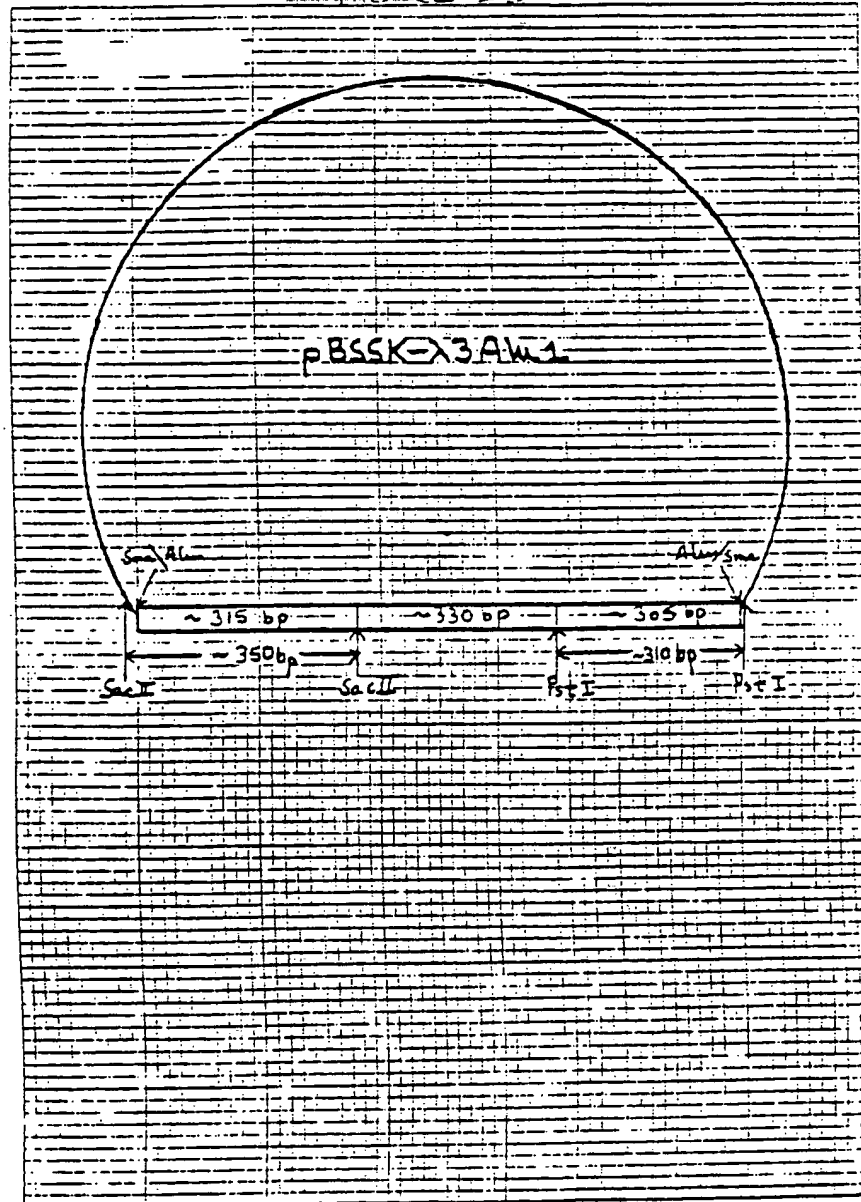
GGA CGG GAC TTT AAG ATG AAG TTA TGG GAT GTC GTG GCT TGC CTG CTG GTG CTG  
 G R D F K M K L W D V V A V C L V L

CTC CAC ACC GCG TCC GCG TTC CCG CTG CCC GCG GGT AAG AGG CCT CCC GAG GCG  
L H T A S A A P L P A G K R P P E A

CCC GCC GAA GAC CGC TCC CTC GGC CGC CGC CGC TTC CGC CTG AGC AGT  
 P A E D R S L G R R A P A L S S

GAC Tgtaagaaccgttcc  
D

FIGURE 23



46 0700

N-E 18.10.00 10.10.00 10.10.00 10.10.00

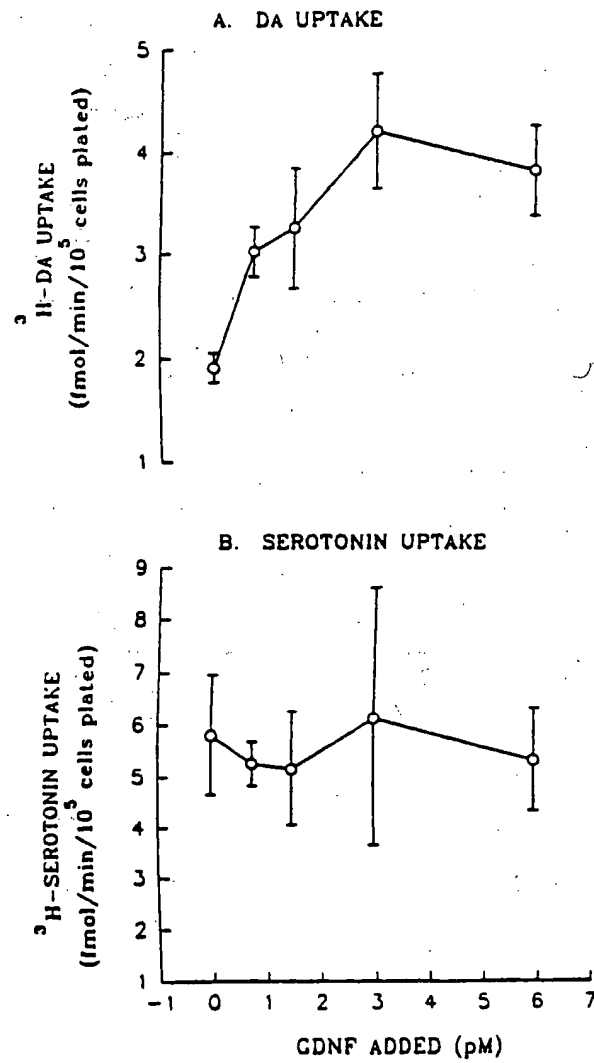


FIGURE 24

FIGURE 25

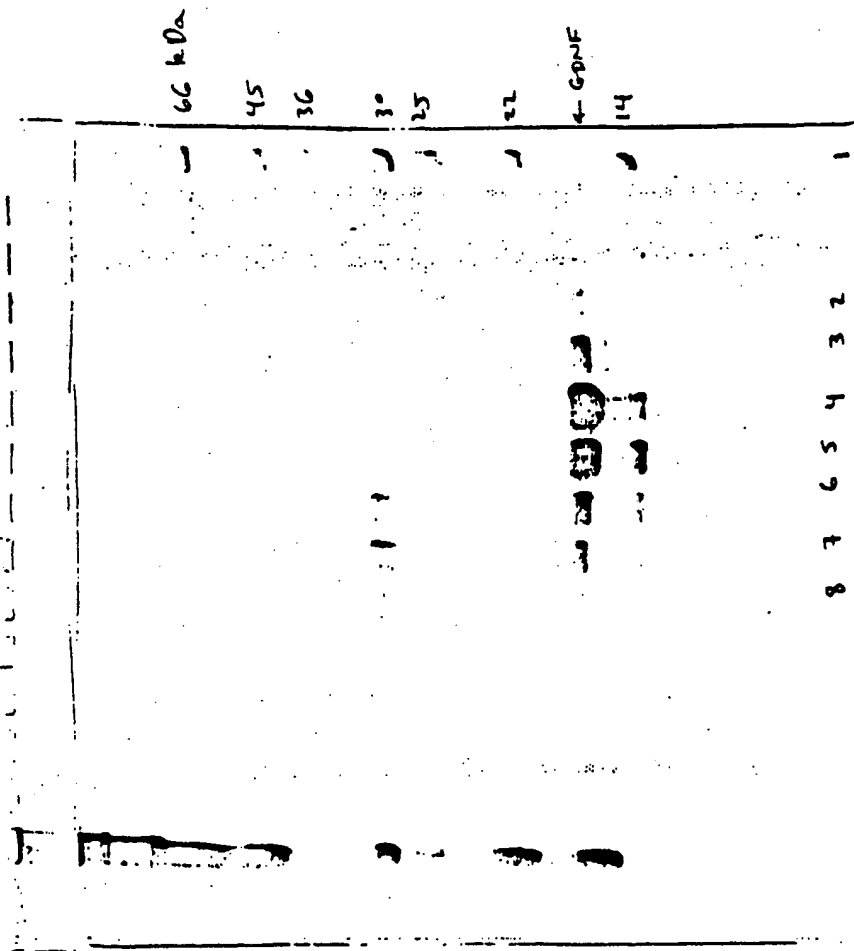


FIGURE 26

